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THE BACTERIAL FLORA AS A FACTOR IN THE UNPRODUCTIVENESS OF SOILS.*

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The splendid contributions concerning the general relationship existing between soil micro-organisms and scientific agriculture are their own testimony as to the soundness of this position. Of these contributions the subject of nitrification is one which has received the larger share of attention from scientific men, and the literature thereon is indeed voluminous. The value of nitrogen fixation by bacteria living within the soil itself and by bacteria which develop nodules upon the roots of leguminous plants, and the consequent increase in fertility of abandoned fields is a fact with which every student of agriculture has become familiar. A role obviously less generally understood or appreciated is that of micro-organisms in rendering a field or a habitat injurious to agricultural crops. Micro-organic life in soils and the relationship of such species as friends or foes to the crop-producing capacity of soils is a line of research still before us. It is one which offers splendid opportunities for the collection of facts of great moment to the practice of agriculture, particularly in relation to the much debated question of fertilizers. It will enable a better economic utilization and conservation of soil resources.

The number of species concerned is exceedingly great. Some are aerobic, while others are anaerobic. There are present not only beneficial nitrifying bacteria upon which the formation of important, valuable chemical compounds in the soil depends, but also denitrifying, putrefactive, and pathogenic bacteria to

*Contribution from the Botanical Laboratory of Ohio State University, 53.

which most of the diseases of the soil may be attributed. The problem concerning the processes and the products of the activity of the injurious bacteria, and the correlated question of their intimate bearing upon a decreased fertility in soils, has unfortunately been limited to work of a comparatively small number of investigators. A glance through the literature of research in soil bacteriology reveals that scarcely anything has been published on the physiological effects of bacterial decomposition products upon agricultural plants.

Recent work of an experimental nature which dealt primarily with physiologically arid habitats and drought resistance in plants (Bot. Gazette **49**: 1910) has revealed to the writer that the injurious products of a bacterial soil flora accumulating in definite layers of soil are the leading factor to be considered in the sterility of certain soils, and that these products operate selectively upon invading forms striving for occupancy. The attempt which has been made to study the physiological reaction of the products formed from the activity of single, isolated species as well as the effects of the residual products due to mixtures of bacteria is briefly stated below. The data have been tabulated and are offered now in the hope that they will be of general interest, and invite other investigators to make studies similar to the one here presented. A more detailed account covering more extensive investigations will appear later.

Without going into too much detail it is sufficient here to point out the following: In the spring of 1908 an examination of bog water and bog soils which was carried on in connection with the physiological ecology of Cranberry Island at Buckeye Lake, Ohio, disclosed that the formation of methane and other gases was of bacterial origin. Agricultural plants and various other cultivated varieties which were grown on Cranberry Island for experimental purposes showed marked difficulty of absorption, soon became stunted, took on xerophilous characters, and in most cases died. Through the courtesy of Prof. Morrey of the Bacteriological Department of this University, the bacterial examination was repeated in 1909. Under Dr. Morrey's direction, Mr. W. L. Sherman, to whom much credit is due for efficient aid, prepared dilution cultures from fresh samples of bog water. The isolation of the various species was continued upon peat-agar plates and later in test-tubes containing a beef-broth-agar medium, until from the bacterial colonies which appeared upon them the pure cultures were obtained. The bacteria thus isolated were tested for their toxin producing power upon a sterilized solution of bog water and peat. A number of large flasks of a liter capacity containing the sterilized solution were inoculated with the respective pure cultures. Several flasks were left sterile to serve as controls, while others were inoculated with

a mixture of bacteria found in 1 cc. of fresh bog water. An additional test condition was arranged at the same time from the normal, untreated bog water. All flasks were then placed in an incubator for a period varying from two to four, and six weeks At such times they were then brought to the Botanical laboratory. All physiological experiments were made in duplicate series and the greatest caution was observed to reduce the dangers of contamination during the preparation of the cultures. The physiological tests were made in half-liter "Mason" jars covered with black paper and containing 500 cc. of the inoculated solution. Wheat seedlings were used for these cultures. The seeds were germinated in sterilized quartz sand until 4–5 cm. high*. They were then carefully washed in distilled water and transplanted to the cultures. Six seedlings were used in every experiment. The seedlings were individuals selected out of a large number of plants. The flat corks to which they were fastened were previously sterilized and paraffined. The cultures were then placed in the University greenhouse in situations where the conditions of temperature and diffused light were uniform. In connection with temperature and humidity readings the measurement of the evaporating power of the air was obtained using for this purpose a standardized porous cup atmometer. The growth of the plants in the various cultures was measured by transpiration relative to the control cultures; the water loss was recorded every fifth day by weighing the cultures. In all cases the experiments were extended for fifteen days. About 35 different species of bacteria have thus far been isolated from the uppermost layer of the soil (to the depth of one foot) and 21 of them have been tested physiologically. From the data at hand the following have been selected to illustrate the variation in virility of bacterial products.

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* The following method, used by the writer for some time. is found to be convenient and very valuable for sprouting wheat seeds. An enameled dish 20 cm. in diameter and 8 cm. high, the bottom of which is perforated with openings of 2 mm. is filled with sterilized quartz sand. To keep the quartz from falling through the dish is lined with filter paper, or the openings are decreased to a size allowing the needed contact with the water by repeated dipping of the dish in melted paraffin. The dish is placed upon cork supports into a large enameled iron pan, 25 x 10 cm., containing water up to the lower 2 cm. of the dish. To prevent injury to the seedlings from the accumulation of materials which the seeds exude during germination the water is changed daily. The germinator is covered with a glass-stoppered bell-jar whose stopper may readily be replaced by one of rubber with one or more holes. When the plants are of the desired height the pan is filled with water thus allowing a ready removal.

TABLE I.

TRANSPIRATION DATA FOR SOLUTIONS INOCULATED NOV. 14, 1909, WITH PURE CULTURES OF BOG BACTERIA.

Series IV	Bacteria	TRANSPIRATION IN GRAMS				Comparative transpiration	Percentage decrease
		5th day (Dec. 16th)	10th day	15th day	Total		
	Check	9.33	42.92	66.85	119.10	100.	0.
	B. 20	8.85	41.30	44.06	94.21	79.10	20.90
	B. 22	8.30	38.15	42.90	89.35	75.02	24.98
	B. 7	8.55	31.80	42.80	83.15	69.81	30.19
	C. 3	7.15	30.90	43.95	82.00	68.85	31.15
	C. 4	7.60	29.70	44.40	81.70	68.59	31.41
Duplicates	Check	8.80	44.50	66.83	120.13	100.	0.
	B. 20	8.40	34.25	45.98	88.63	73.77	26.23
	B. 22	7.05	35.40	46.10	88.55	73.71	26.29
	B. 7	8.15	34.45	42.21	84.81	70.59	29.41
	C. 3	8.10	30.90	44.25	83.25	69.30	30.70
	C. 4	8.40	31.15	41.65	81.20	67.59	32.41
Atmometer		102 grs.	136 grs.	125 grs.			

Using the transpiration of the controls as a basis and representing it as unity the different bacterial cultures have values in the order as indicated in the last two columns of the table. These figures show conclusively that in all cases the bacteria are responsible for the proportionally diminished transpiration and growth. The transpiration values fluctuate to a considerable extent; in some cases the differences from the controls are not so very great, but in all cultures the values lie below that of the control.

The evidence derived from the duplicate series is omitted, showing, as it does, results as closely parallel as in Table I. To what extent Table II suggests the possibility that bacteriological diagnosis when correlated with physiological criteria may determine the crop-producing power of different soils need not be discussed at length. The figures speak for themselves. Several facts, however, seem to be clearly brought out in the above data. The transpiration figures of the first five days in B. 25 and B. 1 cc. indicate that the growth of the plants was considerably stimulated by the presence of the toxic bodies in the solution. Those of the last five days prove that the solution was decidedly injurious. B. 13 is worthy of note since the plants

TABLE II.

TRANSPIRATION DATA FOR SOLUTIONS INOCULATED JAN. 15, 1910, WITH PURE CULTURES OF BOG BACTERIA.

Series VII	Bacteria	TRANSPIRATION IN GRAMS				Comparative transpiration	Percentage decrease
		5th day (Feb. 4th)	10th day	15th day	Total		
	Check	17.65	36.20	36.60	90.45	100.	0.
	Normal bog water	7.65	11.30	8.90	27.85	30.79	69.21
	B. 25	18.15	29.30	26.85	74.30	82.14	17.86
	B. 1 cc.	18.27	30.15	25.70	74.12	81.94	18.06
	B. 13	15.72	24.65	30.85	71.22	78.74	21.26
	B. 2	17.45	29.05	24.30	70.80	78.27	21.73
	B. 1	16.60	28.95	24.85	70.40	77.83	22.17
	B. 27	12.60	24.90	22.80	60.50	66.66	33.34
	B. 6	14.00	25.40	20.80	60.20	66.65	33.45
	B. 4	14.95	23.80	20.45	59.20	65.46	34.54
	B. 29	11.60	15.55	15.85	43.00	47.54	52.46
Atmometer		114 grs.	117 grs.	102 grs.			

in that solution disclose a gradually intensified power of resistance and a physiological phase marked by a greater functional activity. The maximum rate of transpiration occurred on the fifteenth day as in the control, while that of all remaining cultures appeared on the tenth day. As compared with the control the inoculated cultures, it will be observed, have reduced the transpiration quantity of wheat plants from 20% to 52%. Another matter is the degree in which individual plants vary in tolerance and resistance. When the bacteria are omitted from the sterilized solution no evidence of toxicity is noticeable for the wheat plants growing in the solution, and their variability in growth, and green and dry weight deviates but little from the common norm. But when inoculated the culture medium becomes a condition always active in stimulating or depressing normal functions. The task of securing a co-ordination between functions of absorption, transpiration, and transport becomes, indeed, a complicated one for the plants, varying greatly within the same species and with different species. The analysis of these experiments has strengthened the conviction that the best functioning plants rather than the general average represent the proper test of the possibilities of agricultural plants under the given conditions, and that adjustment to conditions is a more

noteworthy characteristic than structural deviations or acclimatization. Much economic value would attach to an extension of these experiments by determining through selection and a more detailed physiological study the cultivated forms resistant and immune to the effects of this type of soil bacteria, and the nature of the resistance.

In order to determine the ability of the micro-organisms to convert soluble proteids into amido-acids and allied products from the decomposition of proteids enough peptone was added to solutions of sterilized bog-water and peat to make an equivalent of a 1% peptone culture. After sterilization the solutions were inoculated with the bacteria indicated in Table III. The cultures were then tested physiologically at the end of a two-weeks incubation. Since the danger of contamination becomes increasingly greater with peptone cultures, the transpiration figures for only the first five days are tabulated. They are believed to be entirely consonant with the true state of affairs since the figures in the duplicate cultures appeared in every way parallel. The wheat plants had grown in each experiment for three days at the time the photographs here added were made for the writer by Prof. Schaffner.

TABLE III.

TRANSPIRATION DATA FOR 1% PEPTONE CULTURE SOLUTIONS INOCULATED JAN. 15, 1910, WITH PURE CULTURES OF BOG BACTERIA.

Series IX	Number	Bacteria	TRANSPIRATION IN GRAMS		
			5th Day (Feb. 4th)	Comparative transpiration	Percentage decrease
	6	Control	17.65	100.	0.
	7	Pep. chk	7.00	39.65	60.35
	13	B. 13	4.85	27.47	72.53
	14	B. 25	2.70	15.30	84.70
	11	B. 2	2.30	13.03	86.97
	12	B. 4	2.40	13.60	86.40
	15	B. 1 cc.	1.87	10.60	89.40

A brief inspection of the figures and the photographs suffices to show that transpiration, growth, green and dry weight of wheat plants are in this case proportionally reduced. Compared with the weekly atmometer readings it is evident that transpiration is not merely a function of absorption and of growth but also a function of the rate of evaporating power of air, that is the saturation deficiency of air. The rate of transpiration is seen to be the product of a co-ordination of factors. It is not due to any single factor but to the cumulative action of several conditions.

At the end of the experiment a chemical examination of the peptone culture solutions, made by Dr. Lyman, indicated the presence of indol, ammonia and various non-volatile products in various proportions. A marked difference was noted in the ability of the different species of bacteria to produce indol and ammonia. The highest quantity of ammonia was produced by B. 13; the least amount was recorded for B. 1 cc.—the culture solution, it will be remembered, which consisted of a mixture of the bacteria found in one cubic centimeter of fresh bog water. None of these products were found in the control (sterilized bog water and peat). It is also to be noted that neither the organic acids nor the ammonia underwent a further change and that the absence of atmorpheric air is not a limiting essential condition for the growth of the bacterial organisms. Interesting is the fact that the organisms belong for the most part to the aerobs. The mixture culture solution (B. 1 cc.) in which the percentage decrease in transpiration was as low as 90%, seems to show that it is the function of some of the bacterial organisms to do the initial work of rendering soluble the protein compounds in the soil. The process of denitrification is carried on up to a point where further decomposition is continued by other organisms. Judging from the differences in the transpiration values of the various cultures, a whole series of bacteria seems therefore to be involved to whom are due the residual products, the algebraic sum of which in part constitutes the toxicity of the habitat encountered on Cranberry Island, the formation of methane gas, and the reactions which form the basis of the process of humification.

Thus far the isolation of bacteria involved in the decomposition of carbohydrates has not been successful. Certain microorganisms have been found to possess the ability to dissolve cellulose (filter paper) in the presence of air. To what extent these forms and the anaerobs play a role in the relation of deleterious products in the soil and cultivation of crops is now under investigation.

It is not proposed to dwell upon the general aspect of this problem in this place. In a previous paper (Botanical Gazette 47: 389–405, 1909) the writer has reported that the poisonous matter injurious to plant growth is present in the agricultural soils used as filters for bog water. The retardation seen in the contaminated soils lacked the corresponding control average in dry weight of plants to an amount of 18 per cent, 3 per cent and 36 per cent. for sand, clay, and humus soils respectively. It was further shown that the absorption and retention capacity of soil for toxins became generally higher the greater the content of humus. In concluding this part of the discussion it is well to

note the extent in which the results show clearly that the retardation in growth of wheat plants is not caused by physical or chemical conditions but through the direct activity of the bacterial flora. It has long been suspected that a reciprocal relation exists between groups of soil bacteria and the plants growing upon the soil. Various writers have been able to point out that marked differences in the productive power of different soils followed the growth of wild plants, and that these differences persist for some time. It is generally concluded therefore, that the injury caused to cultivated plants by weeds or previous crops might be due to influences on the bacterial life in the soil, and in

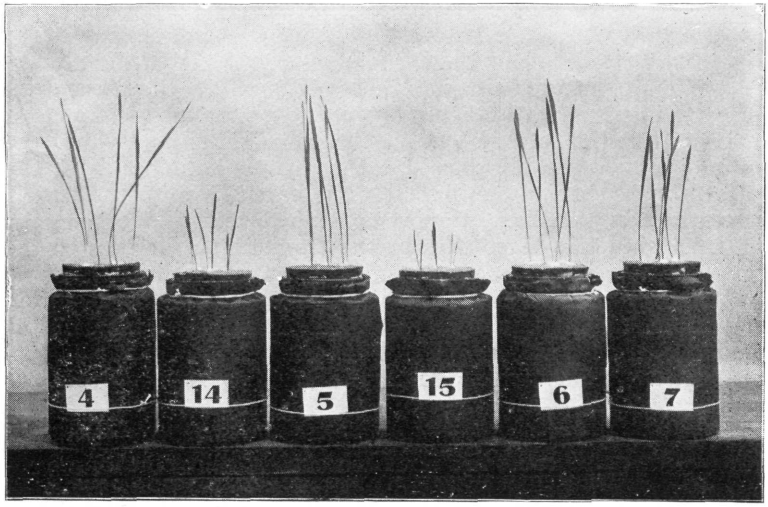

Fig. 1. Wheat plants growing in 1 per cent. peptone bog-water solutions inoculated with pure cultures of bog bacteria. Numbers correspond with data in Table III.

a direction unfavorable to succeeding agricultural crops. That such relations exist the writer is convinced in view of the evidence presented above. No doubt, the "exhaustion" of soils which is frequently met with, and which cannot always be attributed to the removal of plant nutrients, is, in part, an allied phenomenon. It cannot remain a matter of indifference to physiological ecologists whether a strong, intimate, and controlling relation exists between soil bacteria and surface flora, and how the bacterial organisms affect the character, and the association and succession of plants. At best very little is known of this phase of the physiographic process, and of the reactions and effects of the bacterial products upon plant life. It would be idle, also, to expect that the bacteriological data in themselves are sufficient for a clear interpretation of toxicity

and unproductiveness of soils. If attempted, the interpretation would be indeed, one-sided; there is a co-ordination of factors, each and all of which exert a relatively marked influence. Climatic conditions, temperature, water, and air conditions in the soil, as well as the phsycial and chemical character of it, and the surface flora, all play an important role in determining the character of a vegetation and of its bacterial flora, and therefore also the character of the chemical products formed.

One should constantly keep in mind the genetic idea in the study of edaphic, climatic, or biotic investigations. Soil, climate and flora are the product of the conditions of their

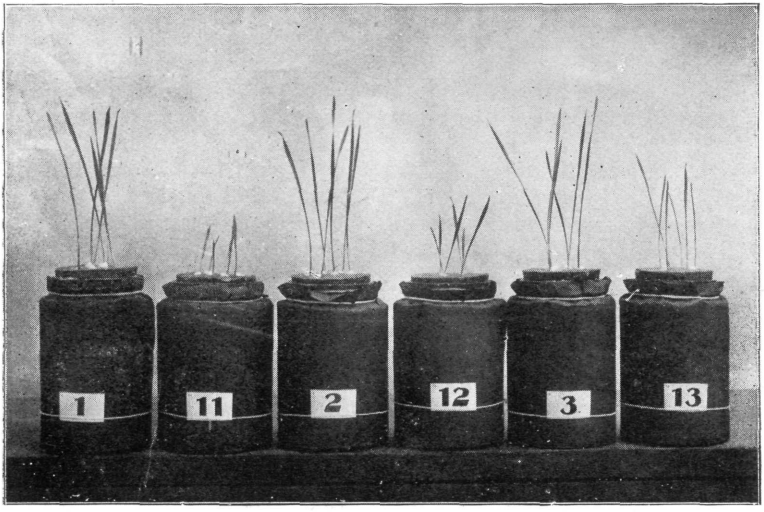

Fig. 2. Wheat plants growing in 1 per cent. peptone bog-water solutions inoculated with pure cultures of bog bacteria. Numbers correspond with data in Table III.

development; their peculiarities are closely interrelated in the dynamics of the process. Wherever the same factors are present, the results will be similar. The bacteriological-chemical, as well as the physiological method, deserve on that account a closer consideration. The determination of the bacterial transformation products and the more detailed study of their physiological properties should possess an exactness and a reliability to make them suitable for the solution not only of agricultural but of ecological problems as well. It is only too clear that the need for new investigations in this phase of the problem is pressing, and that especially new points of view and new lines of research are imperatively required.

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